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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 39/00, 39/385, 39/39

A1

(11) International Publication Number: WO 98/16247

(43) International Publication Date: 23 April 1998 (23.04.98)

(21) International Application Number: PCT/US97/19004

(22) International Filing Date: 9 October 1997 (09.10.97)

(30) Priority Data:
60/028,118 11 October 1996 (11.10.96) US

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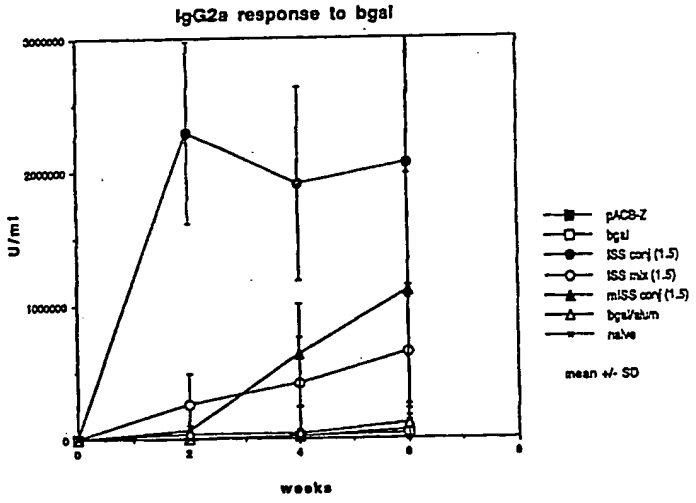
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IMMUNOSTIMULATORY POLYNUCLEOTIDE/IMMUNOMODULATORY MOLECULE CONJUGATES



(57) Abstract

Immunostimulatory polynucleotide–immunomodulatory molecule conjugate compositions are disclosed. These compositions include a polynucleotide that is linked to an immunomodulatory molecule, which molecule comprises an antigen and may further comprise immunomodulators such as cytokines and adjuvants. The polynucleotide portion of the conjugate includes at least one immunostimulatory oligonucleotide nucleotide sequence (ISS). Methods of modulating an immune response upon administration of the polynucleotide–immunomodulatory conjugate preparation to a vertebrate host are also disclosed.

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IMMUNOSTIMULATORY POLYNUCLEOTIDE/IMMUNOMODULATORY MOLECULE CONJUGATES

RELATED U.S. PATENT APPLICATIONS

This is a continuation-in-part and utility conversion of U.S. Provisional Patent Application Serial No. 60/028,118, filed October 11, 1996.

STATEMENT OF FEDERALLY SPONSORED RESEARCH

Support for the research disclosed herein may have been provided by the National Institutes of Health under Grant Nos. AI37305 and/or AR25443.

FIELD OF THE INVENTION

The invention relates to compositions comprising an immunomodulatory molecule (IMM) including an antigen, conjugated to a polynucleotide that contains or consists of at least one immunostimulatory oligonucleotide (ISS-PN). It also relates to methods for modulating the immune response of a vertebrate host to an antigen.

HISTORY OF THE RELATED ART

Conventionally, immunization of a host against an antigen is accomplished by repeatedly vaccinating the host with the antigen. While most current vaccines elicit reasonable antibody responses, cellular responses (in particular, major histocompatibility complex (MHC) class I-restricted cytotoxic T cells) are generally absent or weak. For many infectious diseases, such as tuberculosis and malaria, humoral responses are of little protective value against infection.

Given the weak cellular immune response to protein antigens, modulation of the immune responses to these antigens has clear importance. The ability to modify immune responses to protein or peptide antigen has implications for tumor therapy, for the treatment of allergic disorders and for treatment of other conditions achievable through induction of a vigorous cellular immune response.

SUMMARY OF THE INVENTION

The present invention provides compositions comprising an ISS-PN which is conjugated to an IMM (which includes an antigen) to form ISS-PN/IMM conjugates. The ISS-PN/IMM conjugates of the invention are biological response modifiers in the sense that they modify the humoral and cellular immune response of a host to an antigen.

Specifically, the ISS-PN and IMM components of the ISS-PN/IMM conjugates synergistically boost the magnitude of the host immune response against an antigen to a level greater than the host immune response to either the IMM, antigen or ISS-PN alone. The ISS-PN/IMM conjugates also shift the host cellular immune response away from the helper T lymphocyte type 2 (Th2) phenotype toward a helper T lymphocyte type 1 (Th1) phenotype. These responses to ISS-PN/IMM conjugates are particularly acute during the important early phase of the host immune response to an antigen.

To these ends, ISS-PN/IMM conjugates are delivered by any route through which antigen-sensitized host tissues will be contacted with the ISS-PN/IMM conjugate. ISS-PN/IMM conjugates administered in this fashion boost both humoral (antibody) and cellular (Th1 type) immune responses of the host. Thus, use of the method to boost the immune responsiveness of a host to subsequent challenge by a sensitizing antigen without immunization avoids the risk of Th2-mediated, immunization-induced anaphylaxis by suppressing IgE production in response to the antigen challenge. An especially advantageous use for this aspect of the invention is treatment of localized allergic responses in target tissues where the allergens enter the body, such as the skin and mucosa.

Suppression of the Th2 phenotype according to the invention is also a useful in reducing antigen-stimulated IL-4 and IL-5 production. Thus, the invention encompasses delivery of ISS-PN/IMM conjugates to a host to suppress the Th2

phenotype associated with conventional antigen immunization (e.g., for vaccination or allergy immunotherapy).

The shift to a Th1 phenotype achieved according to the invention is accompanied by increased secretion of IFN α, β and γ, as well as IL-12 and IL-18. Each of these cytokines enhance the host's immune defenses against intracellular pathogens, such as viruses. Thus, the invention encompasses delivery of ISS-PN/IMM conjugates to a host to combat pathogenic infection.

Angiogenesis is also enhanced in the Th1 phenotype (ostensibly through stimulation by IL-12). Thus, the invention encompasses delivery of ISS-PN/IMM conjugates to a host to stimulate therapeutic angiogenesis to treat conditions in which localized blood flow plays a significant etiological role; e.g., retinopathies.

The ISS-PN/IMM conjugates of the invention comprise an IMM conjugated to a polynucleotide that includes, or consists of, at least one immunostimulatory oligonucleotide (ISS-ODN) moiety. The ISS-ODN moiety is a single- or double-stranded DNA or RNA oligonucleotide having at least 6 nucleotide bases which may include, or consist of, a modified oligonucleoside or a sequence of modified nucleosides.

The ISS-ODN moieties comprise, or may be flanked by, a CpG containing nucleotide sequence or a p(IC) nucleotide sequence, which may be palindromic. Where the oligonucleotide moiety comprises a CpG sequence, it may include a hexamer structure consisting of: 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'. Examples of such hexamer structures are AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

In one aspect of the invention, the ISS-PN consists of an ISS-ODN. Alternatively, the ISS-PN comprises an ISS-ODN.

Conjugates of the invention also include PN/IMM wherein the PN serves as a carrier to introduce the IMM antigen into MHC Class I processing pathways not normally stimulated by soluble antigen, but lacks ISS activity and therefore does not stimulate a Th1 phenotype immune response. Examples of such PN/IMM are those wherein the CpG motif is mutated, for example, to a GpG motif.

In one aspect of the invention, the IMM conjugate partner to the ISS-PN consists of an antigen. Such antigens are selected from the group of antigens consisting of proteins, peptides, glycoproteins, polysaccharides and gangliosides.

In another aspect of the invention, the IMM conjugate partner comprises an antigen and further comprises an immunostimulatory molecule selected from the group of such molecules consisting of adjuvants, hormones, growth factors, cytokines, chemokines, targeting protein ligands, and trans-activating factors.

In another aspect of the invention, the ISS-PN/IMM conjugate is modified for targeted delivery by, for example, attachment to a monoclonal antibody, receptor ligand and/or liposome.

Pharmaceutically acceptable compositions of ISS-PN/IMM conjugates are provided for use in practicing the methods of the invention. Where appropriate to the contemplated course of therapy, the ISS-PN/IMM conjugates may be administered with anti-inflammatory or immunotherapeutic agents. Thus, a particularly useful composition for use in practicing the method of the invention is one in which an anti-inflammatory agent (e.g., a glucocorticoid) is mixed with, or further conjugated to, an ISS-PN/IMM conjugate.

The ISS-PN/IMM conjugates can also be provided in the form of a kit comprising ISS-PN/IMM conjugates and any additional medicaments, as well as a device for delivery of the ISS-PN/IMM conjugates to a host tissue and reagents for determining the biological effect of the ISS-PN/IMM conjugates on a treated host.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graph of data demonstrating the vigorous Th1-type immune response (as measured by production of IgG2a against an IMM antigen) stimulated by ISS-PN/IMM (1:5 ratio) in comparison to the levels of Th2-like responses stimulated by an ISS containing, antigen encoding plasmid (pACB-Z); the antigen alone (β-gal); the antigen mixed with an ISS (1:5 ratio); the antigen conjugated to a non-stimulatory PN (mISS conj; 1:5 ratio); the antigen in adjuvant (alum) and, for reference, the IgG2a levels in naive (unexposed) mice. The horizontal axis represents the levels (units/ml) of antibody; the vertical axis represents the number of weeks following primary antigen exposure.

FIGURE 2 is a graph of data demonstrating the levels of Th2-type immune responses (as measured by production of IgG1 against an IMM antigen) stimulated by an ISS containing, antigen encoding plasmid (pACB-Z); the antigen alone (β-gal); the antigen mixed with an ISS (1:5 ratio); the antigen conjugated to a non-stimulatory PN (mISS conj; 1:5 ratio); the antigen in adjuvant (alum) and, for reference, the IgG1 levels in naive (unexposed) mice, all as compared to the vigorous Th1-type immune response produced in mice immunized with ISS-PN/IMM (1:5 ratio). The horizontal axis represents the levels (units/ml) of antibody; the vertical axis represents the number of weeks following primary antigen exposure.

FIGURE 3 is a graph of data demonstrating the vigorous Th1-type immune response (as measured by production of IgG2a against an IMM antigen) stimulated by ISS-PN/IMM in comparison to the levels of Th2-like responses stimulated by the antigen alone (AgE) and antigen conjugated to a non-stimulatory PN (mISS conj). Antigen to PN ratios are all 1:5. The horizontal axis represents the levels (units/ml) of antibody; the vertical axis shows the levels at 4 weeks following primary antigen exposure (shaded bars) and at 2 weeks following secondary antigen challenge (solid bars).

FIGURE 4 is a graph of data demonstrating the levels of Th2-type immune responses (as measured by production of IgG1 against an IMM antigen) stimulated by the antigen alone (AgE) and antigen conjugated to a non-stimulatory PN (mISS conj) in comparison to the vigorous Th1-type immune response stimulated in ISS-PN/IMM immunized mice. Antigen to PN ratios are all 1:5. The horizontal axis represents the levels (units/ml) of antibody; the vertical axis shows the levels at 4 weeks following primary antigen exposure (shaded bars) and at 2 weeks following secondary antigen challenge (solid bars).

FIGURE 5 is a graph of data demonstrating suppression of Th2 associated anti-antigen (AgE) IgE production by ISS-PN/IMM in comparison to the levels of IgE production stimulated by the antigen alone (AgE) and the antigen conjugated to a non-stimulatory PN (mISS conj). Antigen to PN ratios are all 1:5. The horizontal axis represents the levels (counts per minute; cpm) of antibody; the vertical axis shows the levels at 4 weeks following primary antigen exposure (shaded bars) and at 2 weeks following secondary antigen challenge (solid bars).

FIGURE 6 is a graph of data demonstrating the high levels of Th1 associated interferon γ (IFNg) production stimulated by ISS-PN/IMM in comparison to the relatively low levels of the Th1 cytokine stimulated by an ISS containing, antigen encoding plasmid (pACB-Z); the antigen alone (β-gal); the antigen mixed with an ISS; the antigen conjugated to a non-stimulatory PN (mISS conj); the antigen in adjuvant (alum) and, for reference, the IFNg levels in naive (unexposed) mice. Antigen to PN ratios are all 1:5. The horizontal axis represents the levels (ng/ml) of cytokine; the vertical axis shows the levels of cytokine at 4 weeks following primary antigen exposure (shaded bars).

FIGURE 7 is a graph of data demonstrating the vigorous antigen-specific cytotoxic T lymphocyte (CTL) response stimulated by ISS-PN/IMM in comparison to the levels of CTL production stimulated by an ISS containing, antigen encoding plasmid (pACB-

Z); the antigen alone (β-gal); the antigen mixed with an ISS; the antigen conjugated to a non-stimulatory PN (mISS conj); the antigen in adjuvant (alum) and, for reference, the CTL levels in naive (unexposed) mice. Antigen to PN ratios are all 1:5. The horizontal axis represents the levels of antigen-specific cell lysis obtained (as a percentage of control; no antigen); the vertical axis shows the levels of CTL detected at different effector (antigen) to target ratios, from 0:1 to 10:1. The legend identifies how each cell population was treated.

DETAILED DESCRIPTION OF THE INVENTION

A. *Biological Activity of the ISS-PN/IMM Conjugates*

The immune response stimulated by the ISS-PN/IMM conjugates of the invention differs from the vertebrate immune response to conventional vaccination in both magnitude and quality. In the former respect, the host immune response to an antigen is boosted to a level greater than achieved on exposure to an ISS-PN or antigen administered alone or together in an unconjugated form. Thus, one surprising aspect of the invention is that conjugation of an ISS-PN to an antigen-containing IMM produces a synergism between the immunostimulatory activity of the ISS-PN and the immunomodulatory activity of the IMM that immunizes the host to the antigen more effectively than one would predict.

Advantageously, the immune response stimulated according to the invention differs from the immune response of vertebrates to conventional vaccination in that the latter develops in a Th2 phenotype while the former develops in a Th1 phenotype. In this regard, it is helpful to recall that CD4+ lymphocytes generally fall into one of two distinct subsets; i.e., the Th1 and Th2 cells. Th1 cells principally secrete IL-2, IFNγ and TNFβ (the latter two of which mediate macrophage activation and delayed type hypersensitivity) while Th2 cells principally secrete IL-4 (which stimulates production of IgE antibodies), IL-5 (which stimulates granulocyte infiltration of tissue), IL-6 and IL-10. These CD4+ subsets exert a negative influence on one another; i.e., secretion of Th1 lymphokines inhibits secretion of Th2 lymphokines and vice versa.

Factors believed to favor Th1 activation resemble those induced by viral infection and include intracellular pathogens, exposure to IFN-β, IFN-α, IFNγ, IL-12 and IL-18 and exposure to low doses of antigen. Th1 type immune responses also predominate in autoimmune disease. Factors believed to favor Th2 activation include exposure to IL-4 and IL-10, APC activity on the part of B lymphocytes and high doses of antigen.

Active Th1 (IFNγ) cells enhance cellular immunity and are therefore of particular value in responding to intracellular infections, while active Th2 cells enhance antibody production and are therefore of value in responding to extracellular infections (at the risk of anaphylactic events associated with IL-4 stimulated induction of IgE antibody production). Thus, the ability to shift host immune responses from the Th1 to the Th2 repertoire and vice versa has substantial clinical significance for controlling host immunity against antigen challenge (e.g., in infectious and allergic conditions).

To that end, the methods of the invention shift the host immune response to a sensitizing antigen toward a Th1 phenotype (Example I). Consequently, Th2 associated cytokine production and antigen stimulated production of IgE (Examples II and III) are suppressed, thereby reducing the host's risk of prolonged allergic inflammation and minimizing the risk of antigen-induced anaphylaxis. CTL production is also stimulated to a greater degree in animals treated according to the invention. Because CTL production is tied to antigen processing in Class I MHC pathways, increased CTL production can be produced from non-immunostimulatory PN/IMM as well as ISS-PN/IMM (Example IV).

Although the invention is not limited to any particular mechanism of action, it is conceivable that PN facilitate uptake of exogenous antigen by antigen presenting cells for presentation through host MHC Class I processing pathways not normally stimulated by soluble antigen. Thus, ISS-PN/IMM carry antigen into MHC Class I processing pathways (which may also be achieved by PN/IMM without ISS activity) then stimulate a cytokine cascade in a Th1 phenotype (as a result of ISS activity). Whatever the mechanism of action, use of ISS-PN/IMM to boost the host's immune responsiveness to a sensitizing antigen and shift the immune response toward a Th1 phenotype avoids the risk of immunization-induced anaphylaxis, suppresses IgE production in response to a sensitizing antigen and eliminates the need to identify the sensitizing antigen for use in immunization.

With reference to the invention, "boosting of immune responsiveness in a Th1 phenotype" in an ISS-PN/IMM treated host is evidenced by:

(1) a reduction in levels of IL-4 measured before and after antigen-challenge; or detection of lower (or even absent) levels of IL-4 in a treated host as compared to an antigen-primed, or primed and challenged, control;

(2) an increase in levels of IL-12, IL-18 and/or IFN (α, β or γ) before and after antigen challenge; or detection of higher levels of IL-12, IL-18 and/or IFN (α, β or γ) in an ISS-PN/IMM treated host as compared to an antigen-primed or, primed and challenged, control;

(3) IgG2a antibody production in a treated host; or

(4) a reduction in levels of antigen-specific IgE as measured before and after antigen challenge; or detection of lower (or even absent) levels of antigen-specific IgE in an ISS-PN/IMM treated host as compared to an antigen-primed, or primed and challenged, control.

Exemplary methods for determining such values are described further in the Examples.

Thus, the ISS-PN/IMM conjugates of the invention provide relatively safe, effective means of stimulating a robust immune response in a vertebrate host against any antigen.

B. *ISS-PN/IMM Conjugates: Structure and Preparation*

1. ISS-PN root structure

The ISS-ODN base of the ISS-PN/IMM conjugates of the invention includes an oligonucleotide, which may be a part of a larger nucleotide construct such as a

plasmid. The term "polynucleotide" therefore includes oligonucleotides, modified oligonucleotides and oligonucleosides, alone or as part of a larger construct. The polynucleotide may be single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA).

The polynucleotide portion can be linearly or circularly configured, or the oligonucleotide portion can contain both linear and circular segments. Modifications of oligonucleotides include, but are not limited to, modifications of the 3'OH or 5'OH group, modifications of the nucleotide base, modifications of the sugar component, and modifications of the phosphate group.

The oligonucleotide base of ISS-PN/IMM conjugates may comprise ribonucleotides (containing ribose as the only or principal sugar component), deoxyribonucleotides deoxyribose as the principal sugar component), or in accordance with established state-of-the-art modified sugars or sugar analogs may be incorporated in the oligonucleotide of the present invention. Thus, in addition to ribose and deoxyribose, the sugar moiety may be pentose, deoxypentose, hexose, deoxyhexos, glucose, arabinose, xylose, lyxose, and a sugar "analog" cyclopentyl group. The sugar may be in pyranosyl or in a furanosyl form. In the modified oligonucleotides of the present invention the sugar moiety is preferably the furanoside of ribose, deoxyribose, arabinose or 2'-0-methylribose, and the sugar may be attached to the respective heterocyclic bases either in I or J anomeric configuration. The preparation of these sugars or sugar analogs and the respective "nucleosides" wherein such sugars or analogs are attached to a heterocyclic base (nucleic acid base) per se is known, and need not be described here, except to the extent such preparation may pertain to any specific example.

The phosphorous derivative (or modified phosphate group) which may be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention may be a monophosphate, diphosphate, triphosphate, alkylphosphate,

alkanephosphate, phosphoronthioate, phosphorodithioate or the like. The preparation of the above-noted phosphate analogs, and their incorporation into nucleotides, modified nucleotides and oligonucleotides, per se, is also known and need not be described here.

The heterocyclic bases, or nucleic acid bases which are incorporated in the oligonucleotide base of the ISS-PN/IMM conjugates may be the naturally occurring principal purine and pyrimidine bases, (namely uracil or thymine, cytosine, adenine and guanine, as mentioned above), as well as naturally occurring and synthetic modifications of said principal bases. Those skilled in the art will recognize that a large number of "synthetic" non-natural nucleosides comprising various heterocyclic bases and various sugar moieties (and sugar analogs) have become available in the prior art, such that oligonucleotide base of the ISS-PN/IMM conjugates may include one or several heterocyclic bases other than the principal five base components of naturally occurring nucleic acids. Preferably, however, the heterocyclic base in the oligonucleotide base of the ISS-PN/IMM conjugates is selected form uracil-5-yl, cytosin-5-yl, adenin-7-yl, adenin-8-yl, guanin-7-yl, guanin-8-yl, 4-aminopyrrolo [2.3-d] pyrimidin-5-yl, 2-amino-4-oxopyrolo [2,3-d] pyrimidin-5-yl, 2-amino-4-oxopyrrolo [2.3-d] pyrimidin-3-yl groups, where the purines are attached to the sugar moiety of the oligonucleotides via the 9-position, the pyrimidines via the 1-position, the pyrrolopyrimidines via the 7-position and the pyrazolopyrimidines via the 1-position.

Structurally, the root oligonucleotide of the ISS-PN component of ISS-PN/IMM is a non-coding sequence which may include at least one unmethylated CpG motif. The relative position of any CpG sequence in ISS-PN with immunostimulatory activity in certain mammalian species (e.g., rodents) is 5'-CG-3' (i.e., the C is in the 5' position with respect to the G in the 3' position). PN/IMM can be conveniently obtained by substituting the cytosine in the CpG dinucleotide with another nucleotide; a particularly useful substitution is with a guanine to form GpG dinucleotide containing PN.

Some oligonucleotide ISS (ISS-ODN) are known. In such ISS-ODN, the CpG motif is flanked by at least two purine nucleotides (e.g., GA or AA) and at least two pyrimidine nucleotides (5'-Purine-Purine-[C]-[G]-Pyrimidine-Pyrimidine-3'). CpG motif-containing ISS-ODN are believed to stimulate B lymphocyte proliferation (see, e.g., Krieg, *et al.*, *Nature*, 374:546-549, 1995).

The core hexamer structure of the foregoing ISS-PN may be flanked upstream and/or downstream by any number or composition of nucleotides or nucleosides. However, ISS-PN are at least 6 bases in length, and preferably are between 6 and 200 bases in length, to enhance uptake of the ISS-PN/IMM into target tissues. Those of ordinary skill in the art will be familiar with, or can readily identify, reported nucleotide sequences of known ISS-ODN for reference in preparing ISS-PN. For ease of reference in this regard, the following sources are especially helpful:

Yamamoto, *et al.*, *Microbiol.Immunol.*, 36:983 (1992)
Ballas, *et al.*, *J.Immunol.*, 157:1840 (1996)
Klinman, *et al.*, *J.Immunol.*, 158:3635 (1997)
Sato, *et al.*, *Science*, 273:352 (1996)

Each of these articles are incorporated herein by reference for the purpose of illustrating the level of knowledge in the art concerning the nucleotide composition of known ISS-ODN .

In particular, ISS-PN and PN useful in the invention include those which have the following hexameric nucleotide sequences:

1. For ISS-PN, hexamers having "CpG" motifs or, for PN, hexamers having XpY motifs, where X cannot be C if Y is G and vice-versa; and,

2. Inosine and/or uracil substitutions for nucleotides in the foregoing hexamer sequences for use as RNA ISS-ODN.

For example, DNA based ISS-PN useful in the invention include those which have the following hexameric nucleotide sequences:

AACGTT, AGCGTC, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AGCGCT, GACGCT, GGCGCT, TTCGAA, GGCGTT and AACGCC (respectively, SEQ.ID.Nos. 1-18).

RNA based ISS-PN useful in the invention include those which have the following hexameric nucleotide sequences:

AACGUU, AACGpI, AACGpC, AGCGUC, AGCGpI, AGCGpC, GACGCU, GACGCpI, GACGCpC, GACGUU, GACGpI, GACGpC, GACGUC, GACGpI, GACGpC, and poly(I•C) (respectively, SEQ.ID.Nos. 19-33).

The ISS-PN may or may not include palindromic regions. If present, a palindrome may extend only to a CpG motif, if present, in the core hexamer sequence, or may encompass more of the hexamer sequence as well as flanking nucleotide sequences.

In addition, backbone phosphate group modifications (e.g., methylphosphonate, phosphorothioate, phosphoroamidate and phosphorodithioate internucleotide linkages) can confer anti-microbial activity on the ISS-PN and enhance their stability *in vivo*, making them particularly useful in therapeutic applications. A particularly useful phosphate group modification is the conversion to the phosphorothioate or phosphorodithioate forms of ISS-PN. In addition to their potentially anti-microbial

properties, phosphorothioates and phosphorodithioates are more resistant to degradation *in vivo* than their unmodified oligonucleotide counterparts, making the ISS-PN/IMM of the invention more available to the host.

2. IMM conjugate partners.

The oligonucleotide base of the ISS-PN/IMM conjugate is conjugated to an IMM which includes an antigen and may further include an immunomodulatory agent. An "antigen" is a substance that is recognized and bound specifically by an antibody or by a T cell antigen receptor. Antigens can include peptides, proteins, glycoproteins and polysaccharides, including portions thereof and combinations thereof. The antigens can be those found in nature or can be synthetic.

The term "immunomodulatory" as used herein includes immunostimulatory as well as immunosuppressive effects. Immunostimulatory effects include, but are not limited to, those that directly or indirectly enhance cellular or humoral immune responses. Examples of immunostimulatory effects include, but are not limited to, increased antigen-specific antibody production; activation or proliferation of a lymphocyte population such as NK cells, CD4+ T lymphocytes, CD8+ T lymphocytes, macrophages and the like; as well as increased synthesis of Th1 associated immunostimulatory cytokines including, but not limited to, IL-6, IL-12, IL-18, IFN-α,β and γ, TNF-α and the like. Immunosuppressive effects include those that directly or indirectly decrease cellular or humoral immune responses.

Examples of immunosuppressive effects include, but are not limited to, a reduction in antigen- specific antibody production such as reduced IgE production; activation of lymphocyte or other cell populations that have immunosuppressive activities such as those that result in immune tolerance; and increased synthesis of cytokines that have suppressive effects toward certain cellular functions. One example of this is IFN-γ, which can block IL-4 induced class switch to IgE and IgG1, thereby reducing the

levels of these antibody subclasses.

Thus, an "immunomodulatory agent" suitable for use as conjugate partners for ISS-PN/IMM can be a peptide, such as an antigen or cytokine. Where the ISS-PN/IMM conjugate partner is a peptide, suitable peptides include purified native peptides, synthetic peptides, recombinant proteins, crude protein extracts, attenuated or inactivated viruses, cells, micro-organisms, or fragments of such peptides.

Protein antigens that can serve as IMM conjugate partners include antigens from a wide variety of sources, including allergens such as plant pollens, dust mite proteins, animal dander, saliva, and fungal spores as well as infectious microorganims. Examples of the latter include attenuated or inactivated viruses such as HIV-1, HIV-2, hepatitis, herpes simplex, rotavirus, polio virus, measles virus, human and bovine papilloma virus, and slow brain viruses. For immunization against tumor formation, the conjugate can include tumor cells (live or irradiated), tumor cell extracts, or protein subunits of tumor antigens. Vaccines for immuno-based contraception can be formed by including sperm proteins as the peptide portion of the conjugate.

Among the suitable cytokines for use as components of IMM conjugate partners are the interleukins (IL-1, IL-2, IL-3, etc.), interferons (e.g., IFN-α, IFN-β, IFN-γ), erythropoietin, colony stimulating factors (e.g., G-CSF, M-CSF, GM-CSF) and TNF-α.

IMM conjugate partners can also include amino acid sequences that mediate protein binding to a specific receptor or that mediate targeting to a specific cell type or tissue. Examples include, but are not limited to, antibodies or antibody fragments; peptide hormones such as human growth hormone; and enzymes. Co-stimulatory molecules such as B7 (CD80), trans-activating proteins such as transcription factors, chemokines such as macrophage chemotactic protein (MCP) and other chemoattractant or chemotactic peptides are also useful peptide-based conjugate partners.

More specifically, suitable antigens for use as ISS-PN/IMM conjugate partners include any molecule capable of being conjugated to an oligonucleotide and eliciting a B cell or T cell antigen-specific response. Preferably, antigens elicit an antibody response specific for the antigen. A wide variety of molecules are antigens. These include, but are not limited to, sugars, lipids, autacoids and hormones, as well as macromolecules such as complex carbohydrates, and phospholipids. Small molecules may need to be haptenized in order to be rendered antigenic.

Preferably the antigens are peptides, polysaccharides (such as the capsular polysaccharides used in *Haemophilus influenza* vaccines), gangliosides and glycoproteins. The antigen may be an intact antigen or T cell epitope(s) of an antigen. These can be obtained through several methods known in the art, including isolation and synthesis using chemical and enzymatic methods. In certain cases, such as for many sterols fatty acids and phospholipids, the antigenic portions are commercially available.

Many antigenic peptides and proteins are known in, and available to the art; others can be identified using conventional techniques. Examples of known antigens include, but are not limited to :

a. Allergens such as reactive major dust mite allergens *Der pI* and *Der pII* (*see*, Chua, *et al.*, *J.Exp.Med.*, 167:175-182, 1988; and, Chua, *et al.*, *Int.Arch.Allergy Appl. Immunol.*, 91:124-129, 1990), T cell epitope peptides of the *Der pII* allergen (*see*, Joost van Neerven, *et al.*, *J.Immunol.*, 151:2326-2335, 1993), the highly abundant Antigen E (*Amb aI*) ragweed pollen allergen (*see*, Rafnar, *et al.*, *J.Biol.Chem.*, 266:1229-1236, 1991), phospholipase A_2 (bee venom) allergen and T cell epitopes therein (*see*, Dhillon, *et al.*, *J.Allergy Clin.Immunol.*, __:42-__, 1992), white birch pollen (*Betvl*) (*see*, Breiteneder, *et al.*, *EMBO*, 8:1935-1938, 1989), the *Fel dI* major domestic cat allergen (*see*, Rogers, *et al.*, *Mol.Immunol.*, 30:559-568, 1993), tree pollen (*see*, Elsayed et al., *Scand. J. Clin. Lab. Invest. Suppl.*, 204:17-31,

1991) and grass pollen (*see*, Malley, *J. Reprod. Immunol.*, 16:173-86, 1989).

b. Live, attenuated and inactivated microorganisms such as inactivated polio virus (Jiang *et al.*, *J. Biol. Stand.*, 14:103-9, 1986), attenuated strains of Hepatitis A virus (Bradley *et al.*, *J. Med. Virol.*, 14:373-86, 1984), attenuated measles virus (James *et al.*, *N. Engl. J. Med.*, 332:1262-6, 1995) and epitopes of pertussis virus (e.g., ACEL-IMUNE® acellular DTP, Wyeth-Lederle Vaccines and Pediatrics).

c. Contraceptive antigens such as human sperm protein (Lea *et al.*, *Biochim. Biophys. Acta*, 1307:263, 1996).

The published sequence data and methods for isolation and synthesis of the antigens described in these articles are incorporated herein by this reference to illustrate knowledge in the art regarding useful antigen sources. Those of ordinary skill in the art will be familiar with, or can readily ascertain, the identity of other useful antigens for use as ISS-PN/IMM conjugate partners.

Particularly useful immunostimulatory peptides for inclusion in IMM are those which stimulate Th1 immune responses, such as IL-12 (Bliss, *et al.*, *J.Immunol.*, 156:887-894, 1996), IL-18, INF-α,β and γ or TGF-α. Conjugation of adjuvants (such as keyhole limpet hemocyanin, KLH) to the ISS-PN/IMM conjugate can further enhance the activity of the ISS-PN/IMM conjugates of the invention.

Other useful adjuvants include cholera toxin, procholeragenoid, cholera toxin B subunit and fungal polysaccharides including, but not limited to, schizophyllan, muramyl dipeptide, muramyl dipeptide derivatives, phorbol esters, microspheres, non-Helicobacter pylori bacterial lysates, labile toxin of Escherichia coli, block polymers, saponins, and ISCOMs. For additional adjuvants, those of ordinary skill in the art may also refer to, for example, Azuma, I., "Synthetic Immunoadjuvants: Application to Non-Specific Host Stimulation and Potentiation of Vaccine Immunogenicity"

CLAIMS

1. An immunomodulatory composition comprising an immunomodulatory molecule, which molecule comprises an antigen, conjugated to an polynucleotide that contains at least one immunostimulatory nucleotide sequence (ISS).

2. The composition of claim 1, wherein the antigen is selected from the group consisting of proteins, glycoproteins, polysaccharides and gangliosides.

3. The composition of claim 2, wherein the ISS comprises a nucleotide sequence selected from the group CpG, p(GC) and p(IC).

4. The composition of claim 2, wherein the ISS comprises a CG containing oligonucleotide.

5. The composition of claim 4, wherein the ISS further comprises a pG nucleotide sequence.

6. The composition of claim 4, wherein the CG containing oligonucleotide has the sequence 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'.

7. The composition of claim 3, wherein the CpG, p(GC) or p(IC) containing nucleotide sequence is a palindromic double-stranded or non-palindromic single-stranded oligonucleotide.

8. The composition of claim 6, wherein the oligonucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AACGCT, AGCGCT, GACGCT, and GGCGCT.

9. The composition of claim 6, wherein the oligonucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

10. The composition of claim 6, wherein the oligonucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, and GACGTT.

11. The composition of claim 2, wherein the polynucleotide further comprises a linear DNA sequence.

12. The composition of claim 2, wherein the polynucleotide further comprises a circular DNA sequence.

13. The composition of claim 2, wherein the polynucleotide further comprises an RNA nucleotide sequence.

14. The composition of claim 13, wherein the RNA nucleotide sequence comprises a sequence selected from the group consisting of AACGUU, AACGpI, AACGpC, AGCGUC, AGCGpI, AGCGpC, GACGCU, GACGCpI, GACGCpC, GACGUU, GACGpI, GACGpC, GACGUC, GACGpI, GACGpC.

15. The composition of claim 13, wherein the RNA nucleotide sequence comprises a double-stranded poly(I•C) sequence.

16. The composition of claim 13, wherein the RNA nucleotide sequence comprises a sequence selected from the group consisting of AACGUU, AACGpI, AACGpC, AGCGUC, AGCGpI, AGCGpC.

17. The composition of claim 13, wherein the RNA nucleotide sequence comprises a sequence selected from the group consisting of AACGUU, AACGpI, AACGpC.

18. The composition of claim 2, wherein the polynucleotide further comprises at least one modified oligonucleotide.

19. The composition of claim 11, wherein the ISS is contained within the linear DNA sequence, and further wherein the ISS comprises a Purine, Purine, CG, Pyrimidine, Pyrimidine nucleotide sequence.

20. The composition of claim 11, wherein the ISS is contained within the linear DNA sequence, and further wherein the ISS comprises a CG containing pG nucleotide sequence.

21. The composition of claim 12, wherein the ISS is contained within the circular DNA nucleotide sequence, and further wherein the ISS comprises a Purine, Purine, CG, Pyrimidine, Pyrimidine nucleotide sequence.

22. The composition of claim 12, wherein the ISS is contained within the circular DNA nucleotide sequence, and further wherein the ISS comprises a CG containing pG nucleotide sequence.

23. The composition of claim 13, wherein the ISS is contained within the RNA nucleotide sequence, and further wherein the ISS comprises a Purine, Purine, CG, Pyrimidine, Pyrimidine nucleotide sequence.

24. The composition of claim 13, wherein the ISS is contained with the RNA nucleotide sequence, and further wherein the ISS comprises CG containing pG nucleotide sequence.

25. The composition of claim 4, wherein the CG containing nucleotide sequence further comprises a modified oligonucleotide.

26. The composition of claim 6, wherein the 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3' nucleotide sequence further comprises a modified oligonucleotide.

27. An immunomodulatory composition comprising an immunomodulatory molecule, which molecule comprises an antigen and an immunostimulatory peptide, conjugated to an polynucleotide that contains at least one ISS.

28. The composition of claim 27, wherein the polynucleotide is DNA or RNA.

29. The composition of claim 27, wherein the immunostimulatory peptide is selected from the group consisting of co-stimulatory molecules, cytokines, chemokines, targeting protein ligands, and trans-activating factors.

30. The composition of claim 27, wherein the ISS comprises a DNA or RNA nucleotide sequence selected from the group CG, p(GC) and p(IC).

31. The composition of claim 27, wherein the ISS comprises a CG containing oligonucleotide.

32. The composition of claim 31, wherein the ISS further comprises a pG nucleotide sequence.

33. The composition of claim 31, wherein the CG containing nucleotide sequence is the nucleotide sequence 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'.

34. The composition of claim 31, wherein the CG containing nucleotide sequence is a palindromic double-stranded or non-palindromic single-stranded oligonucleotide.

35. The composition of claim 33, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AACGCT, AGCGCT, GACGCT, and GGCGCT.

36. The composition of claim 33, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

37. The composition of claim 33, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, and GACGTT.

38. The composition of claim 29, wherein the polynucleotide further comprises a linear DNA nucleotide sequence.

39. The composition of claim 29, wherein the polynucleotide further comprises a circular DNA nucleotide sequence.

40. The composition of claim 29, wherein the polynucleotide portion further comprises an RNA nucleotide sequence.

41. The composition of claim 40, wherein the RNA nucleotide sequence comprises a nucleotide sequence selected from the group consisting of AACGUU, AACGpI, AACGpC, AGCGUC, AGCGpI, AGCGpC, GACGCU, GACGCpI, GACGCpC, GACGUU, GACGpI, GACGpC, GACGUC, GACGpI, GACGpC.

42. The composition of claim 40, wherein the RNA nucleotide sequence comprises a double-stranded poly(I•C) nucleotide sequence.

43. The composition of claim 40, wherein the RNA nucleotide sequence comprises a nucleotide sequence selected from the group consisting of AACGUU, AACGpI, AACGpC, AGCGUC, AGCGpI, AGCGpC.

44. The composition of claim 40, wherein the RNA nucleotide sequence comprises a nucleotide sequence selected from the group consisting of AACGUU, AACGpI, AACGpC.

45. The composition of claim 29, wherein the polynucleotide portion further comprises at least one modified oligonucleotide.

46. The composition of claim 38, wherein the ISS is contained within the linear DNA nucleotide sequence, and further wherein the ISS comprises a Purine, Purine, CG, Pyrimidine, Pyrimidine nucleotide sequence.

47. The composition of claim 38, wherein the ISS is contained within the linear DNA nucleotide sequence, and further wherein the ISS comprises a CG containing pG nucleotide sequence.

48. The composition of claim 39, wherein the ISS is contained within the circular DNA nucleotide sequence, and further wherein the ISS comprises a Purine, Purine, CG, Pyrimidine, Pyrimidine nucleotide sequence.

49. The composition of claim 39, wherein the ISS is contained within the circular DNA nucleotide sequence, and further wherein the ISS comprises a CG containing pG nucleotide sequence.

50. The composition of claim 40, wherein the ISS is contained within the RNA nucleotide sequence, and further wherein the ISS comprises a Purine, Purine, CG, Pyrimidine, Pyrimidine nucleotide sequence.

51. The composition of claim 40, wherein the ISS is contained with the RNA nucleotide sequence, and further wherein the ISS comprises CG containing pG nucleotide sequence.

52. The composition of claim 31, wherein the CG containing nucleotide sequence further comprises a modified oligonucleotide.

53. The composition of claim 33, wherein the 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3' nucleotide sequence further comprises a modified oligonucleotide.

54. A method of modulating an immune response comprising the administration of an immunomodulatory composition comprising an immunomodulatory molecule, which molecule comprises an antigen, conjugated to an polynucleotide that contains at least one ISS.

55. The method of claim 54, wherein the route of administration is a dermal route.

56. The method of claim 54, wherein the route of administration is low-frequency ultrasonic delivery.

57. The method of claim 54, wherein the antigen is selected from the group consisting of proteins, glycoproteins, polysaccharides and gangliosides.

58. The method of claim 57, wherein the ISS comprises a DNA or RNA nucleotide sequence selected from the group CG, p(GC) and p(IC).

59. The method of claim 57, wherein the ISS comprises a CG containing oligonucleotide.

60. The method of claim 59, wherein the ISS further comprises a pG nucleotide sequence.

61. The method of claim 59, wherein the CG containing nucleotide sequence is the nucleotide sequence 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'.

62. The method of claim 59, wherein the CG containing nucleotide sequence is a palindromic or non-palindromic oligonucleotide nucleotide sequence.

63. The method of claim 59, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AACGCT, AGCGCT, GACGCT, and GGCGCT.

64. The method of claim 59, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

65. The method of claim 59, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, and GACGTT.

66. The method of claim 54, wherein the immune response modulation comprises the induction of a Th1 response.

67. The method of claim 66, wherein the antigen molecule is selected from the group consisting of proteins, glycoproteins and polysaccharides.

68. The method of claim 67, wherein the ISS comprises a DNA or RNA nucleotide sequence selected from the group CG, p(GC) and p(IC).

69. The method of claim 67, wherein the ISS comprises a CG containing oligonucleotide.

70. The method of claim 69, wherein the ISS further comprises a pG nucleotide sequence.

71. The method of claim 69, wherein the CG containing nucleotide sequence is the nucleotide sequence 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'.

72. The method of claim 69, wherein the CG containing nucleotide sequence is a double-stranded palindromic or single-stranded non-palindromic oligonucleotide nucleotide sequence.

73. The method of claim 69, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AACGCT, AGCGCT, GACGCT, and GGCGCT.

74. The method of claim 69, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

75. The method of claim 69, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, and GACGTT.

76. A method of modulating an immune response comprising the administration of an immunomodulatory composition comprising an immunomodulatory molecule, which molecule is comprised of an antigen and an immunostimulatory peptide, conjugated to an polynucleotide that contains at least one ISS.

77. The method of claim 76, wherein the route of administration is a dermal route.

78. The method of claim 76, wherein the route of administration is low-frequency ultrasonic delivery.

79. The method of claim 76, wherein the immunostimulatory peptide is selected from the group consisting of co-stimulatory molecules, cytokines, chemokines, targeting protein ligands, and trans-activating factors.

80. The method of claim 79, wherein the ISS comprises a nucleotide sequence selected from the group CG, p(GC) and p(IC).

81. The method of claim 79, wherein the ISS comprises a CG containing oligonucleotide.

82. The method of claim 81, wherein the ISS further comprises a pG nucleotide sequence.

83. The method of claim 81, wherein the CG containing nucleotide sequence is the nucleotide sequence 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'.

84. The method of claim 81, wherein the CG containing nucleotide sequence is a double-stranded palindromic or single-stranded non-palindromic oligonucleotide nucleotide sequence.

85. The method of claim 81, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AACGCT, AGCGCT, GACGCT, and GGCGCT.

86. The method of claim 81, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

87. The method of claim 81, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, and GACGTT.

88. The method of claim 76, wherein the immune response modulation comprises the induction of a Th1 response.

89. The method of claim 88, wherein the antigen is selected from the group consisting of proteins, glycoproteins and polysaccharides.

90. The method of claim 89, wherein the ISS comprises a nucleotide sequence selected from the group CG, p(GC) and p(IC).

91. The method of claim 89, wherein the ISS comprises a CG containing oligonucleotide.

92. The method of claim 91, wherein the ISS further comprises a pG nucleotide sequence.

93. The method of claim 91, wherein the CG containing nucleotide sequence is the nucleotide sequence 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'.

94. The method of claim 91, wherein the CG containing nucleotide sequence is a double-stranded palindromic or single-stranded non-palindromic oligonucleotide nucleotide sequence.

95. The method of claim 91, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AACGCT, AGCGCT, GACGCT, and GGCGCT.

96. The method of claim 91, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

97. The method of claim 91, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, and GACGTT.

98. A method for introducing a soluble antigen into the Class I MHC processing pathway of the mammalian immune system to elicit a CTL response to the antigen comprising administering a polynucleotide conjugated to an immunomodulatory molecule, which molecule comprises the antigen, to a mammalian host.

99. The method of claim 98 wherein the polynucleotide includes at least one ISS.

100. The method of claim 98 wherein the polynucleotide is free of ISS.

101. The method of claim 98, wherein the antigen is selected from the group consisting of proteins, glycoproteins and polysaccharides.

102. The method of claim 98, wherein the ISS comprises a nucleotide sequence selected from the group CG, p(GC) and p(IC).

103. The method of claim 98, wherein the ISS comprises a CG containing oligonucleotide.

104. The method of claim 103, wherein the ISS further comprises a pG nucleotide sequence.

105. The method of claim 103, wherein the CG containing nucleotide sequence is the nucleotide sequence 5'-Purine, Purine, CG, Pyrimidine, Pyrimidine-3'.

106. The method of claim 103, wherein the CG containing nucleotide sequence is a double-stranded palindromic or single-stranded non-palindromic oligonucleotide nucleotide sequence.

107. The method of claim 102, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AACGCT, AGCGCT, GACGCT, and GGCGCT.

108. The method of claim 102, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, GACGTT, GGCGTT, AACGTC, and AGCGTC.

109. The method of claim 102, wherein the nucleotide sequence is selected from the group consisting of AACGTT, AGCGTT, and GACGTT.

110. The method of claim 98 wherein the polynucleotide comprises a GpG oligonucleotide.

111. The method of claim 110, wherein the nucleotide sequence is selected from the group consisting of AAGGTT, AGGGTT, GAGGTT, GGGGTT, AAGGTC, AGGGTC, GAGGTC, GGGGTC, AAGGCC, AGGGCC, GAGGCC, GGGGCC, AAGGCT, AGGGCT, GAGGCT, and GGGGCT.

112. The composition of claim 110, wherein the nucleotide sequence is selected from the group consisting of AAGGTT, AGGGTT, GAGGTT, GGGGTT, AAGGTC, and AGGGTC.

113. The composition of claim 110, wherein the nucleotide sequence is selected from the group consisting of AAGGTT, AGGGTT, and GAGGTT.

114. A composition for introducing a soluble antigen into the Class I MHC processing pathway of the mammalian immune system to elicit a CTL response to the antigen comprising a polynucleotide conjugated to an immunomodulatory molecule, which molecule comprises the antigen.

115. The composition of claim 114, wherein the antigen is selected from the group consisting of proteins, glycoproteins and polysaccharides.

116. The composition of claim 114 wherein the polynucleotide comprises a GpG oligonucleotide.

117. The composition of claim 116, wherein the nucleotide sequence is selected from the group consisting of AAGGTT, AGGGTT, GAGGTT, GGGGTT, AAGGTC, AGGGTC, GAGGTC, GGGGTC, AAGGCC, AGGGCC, GAGGCC, GGGGCC, AAGGCT, AGGGCT, GAGGCT, and GGGGCT.

118. The composition of claim 116, wherein the nucleotide sequence is selected from the group consisting of AAGGTT, AGGGTT, GAGGTT, GGGGTT, AAGGTC, and AGGGTC.

119. The composition of claim 116, wherein the nucleotide sequence is selected from the group consisting of AAGGTT, AGGGTT, and GAGGTT.

INTERNATIONAL SEARCH REPORT

Interna tal Application No
PCT/US 97/19004

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 A61K39/385 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 02555 A (UNIV IOWA RES FOUND) 1 February 1996 see page 7, line 5 - page 8, line 6 see page 11, line 10 - line 20; table 1 see page 21, line 18 - line 21	1,54
Y	---	2-53, 55-119
Y	IVAN M. ROIT: "ENCYCLOPEDIA OF IMMUNOLOGY" 1992 , ACADEMIC PRESS , LONDON XP002058362 see page 28 - page 30 see page 30, left column, first paragraph ---	2-53, 55-119
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 1998	25. 03. 1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas,F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 97/19004

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RAZ E. ET AL: "Potential role of immunostimulatory DNA sequences (ISS) in genetic immunization and autoimmunity" ARTHRITIS & RHEUMATISM, vol. 39, no. 9, September 1996, page 615 XP002058356 see the whole document ---	1-119
A	SATO Y. ET AL: "Immunostimulatory DNA sequences necessary for effective intradermal gene immunization" SCIENCE, vol. 273, July 1996, LANCASTER, PA US, XP002058357 see the whole document ---	1-119
A	ARTHUR M. KRIEG ET AL: "CpG motifs in bacterial DNA trigger direct B-cell activation" NATURE, vol. 374, 1995, LONDON GB, pages 546-549, XP002058358 see the whole document ---	1-119
A	BALLAS, Z.K. ET AL: "Induction of NK activity in murine and human cells by CpG motifs in oligodeoxynucleotides and bacterial DNA" JOURNAL OF IMMUNOLOGY, vol. 157, September 1996, BALTIMORE US, pages 1840-1845, XP002058359 see the whole document ---	1-119
A	RAZ E. ET AL: "Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, May 1996, WASHINGTON US, pages 5141-5145, XP002058360 see the whole document ---	1-119
A	BRANDA R.F. ET AL: "Amplification of antibody production by phosphorothioate oligodeoxynucleotides" THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, vol. 128, no. 3, September 1996, pages 329-338, XP002058361 see the whole document ---	1-119
A	WO 95 26204 A (ISIS PHARMACEUTICALS INC) 5 October 1995 see the whole document --- -/--	1-119

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/19004

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3 906 092 A (HILLEMAN MAURICE R ET AL) 16 September 1975 see the whole document ---	1-119
A	US 3 725 545 A (MAES R) 3 April 1973 see the whole document ---	1-119
A	GB 1 234 718 A (MERCK) 9 June 1971 see the whole document -----	1-119

3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/19004

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 112-113 (partially)

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 112-113 refer to the compositions of claim 110; however, claim 110 is a method claim. This is obscure. Hence, claims 112-113 have been understood as method claims.

Remark : Although claims 54-113
are directed to a method of treatment of
the human/animal body , the search has been carried out and based on the alleged effects of the composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna tal Application No
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